8-6-2019

How to Untie a Protein Knot.

Sitao Yin
Thomas Jefferson University

Brittiny Dhital
Thomas Jefferson University

Ya-Ming Hou
Thomas Jefferson University

How to Untie a Protein Knot

Sitao Yin, Brittiny Dhital, and Ya-Ming Hou*

Department of Biochemistry and Molecular Biology, Thomas Jefferson University

Philadelphia, PA 19107

#Corresponding author: ya-ming.hou@jefferson.edu

Running title: Unfolding a protein knot

Summary

The origin of protein backbone threading through a topological knot remains elusive. To understand the evolutionary origin of protein knots, Ko et al (Structure, 2019) used circular permutation to untie a knotted protein. They showed that a domain-swapped dimer releases the knot and the associated high-energy state for substrate binding.

Preview

Proteins with a topological knot represent a subset of complex structures that play important roles in biology, such as promoting efficient catalysis of certain enzyme reactions. Despite the increasing number of knotted protein structures being deposited to databases, the evolutionary origin of these protein knots is unclear. One mechanism suggested by an earlier bioinformatics analysis was circular permutation (Tkaczuk et al., 2007), in which an ancestor protein without a topological knot was circularly changed in the order of amino acids to create new termini with rearranged connectivity and spatial orientation of secondary structural elements to form a knotted progeny. While natural proteins related by circular permutations are known as a result of gene duplication (Goldenberg and Creighton, 1983; Lo et al., 2012), there has been no example of a knotted protein derived from an unknotted protein by circular permutation through the process of evolution or protein engineering. Hsu and colleagues now report a successful design of circular permutation (Ko et al., 2019), which converted a knotted protein to an unknotted protein by closing the original N and C termini and introducing a circular permutation site that opens the knotting loop (Figure 1A). In the new structure (Figure 1B), the original topological knot has been untied and the mechanical constraint associated with the knot has been released, resulting in a relaxed configuration that no longer binds the substrate. This work suggests the intriguing possibility that a protein knot was developed in evolution to address a specific structure-function demand for substrate binding.

Hsu and colleagues studied *E. coli* YbeA as an example (Ko et al., 2019), which is a dimeric enzyme belonging to the SPOUT methyl transferase family that uses a conserved trefoil-knot to bind the methyl donor *S*-adenosyl methionine (AdoMet) and to synthesize the methylated $m^3\Psi$ in 23S rRNA (Purta et al., 2008). In the trefoil-knot fold of YbeA, the polypeptide chain makes three crossings in and out of a knotting loop as part of the catalytic site (Mallam et al., 2010). Based on suggestions provided by the circular permutation design server CPred (Lo et al.,

2012), a total of 9 variants of YbeA were made, each with a new opening site near the trefoil-knot. Only the variant CP74, which contains the new opening site at position Ile74 located in the knotting loop (Figure 1C), was soluble upon expression in *E. coli*, indicating its ability to fold into a well-defined structure. Subsequent crystal structural analysis of CP74 by Ko et al. (2019) revealed a dimer that has rearranged specific domains within the original dimer of the native YbeA enzyme. In the CP74 dimer, the C-terminal β strand of one monomer is inserted into the cleft between two N-terminal β strands of the other monomer to stabilize a dimer interface that is substantially expanded relative to the native structure (Figure 1D). In this domain-swapped dimer of CP74, the AdoMet-binding pocket has undergone a major conformational rearrangement, resulting in the loss of the structural complementarity that is necessary to form an appropriate hydrogen-bonding network to accommodate the methyl donor. Indeed, CP74 has no detectable binding affinity to a derivative of the methyl donor, whereas the native enzyme binds the derivative with a low K_d in the μM range, typical of a SPOUT methyl transferase (Christian et al., 2010). Thus, while the resulting domain-swapped variant CP74 possesses a well-defined structure, albeit distinct from the native enzyme, due to the introduction of the circular permutation site to the knotting loop, it has lost the topological knot and no longer binds the methyl donor.

The topological trefoil-knot of YbeA is similar to that in *E. coli* TrmD, a related SPOUT methyl transferase that catalyzes biosynthesis of the methylated m^1G37 in tRNA. In TrmD, the trefoil-knot is required to position AdoMet in the catalytically active form (Christian et al., 2016). Mutations that interfere with the folding of the knot prevent AdoMet from stable binding to the active site and severely compromise the methyl transferase activity. The catalytically active form of AdoMet in TrmD is an unusual “bent” conformation (Christian et al., 2016), in which the adenosine and methionine chemical moieties of the methyl donor fold back to face each other, in contrast to the “open” conformation commonly observed with non-SPOUT methyl

transferases, where the two moieties are splayed out from each other. It is in this bent conformation that AdoMet binding to TrmD facilitates tRNA binding and then helps to assemble the active site for methyl transfer (Christian et al., 2016). Thus, the trefoil-knot fold is a pre-requisite for AdoMet binding in the bent form, which in turn is a pre-requisite for supporting methyl transfer. This catalytic pathway, starting from AdoMet binding in the bent form to the TrmD trefoil-knot to mediating methyl transfer, is likely shared in common with YbeA (Koh et al., 2017). Free energy calculations indicate that the bent form of AdoMet in TrmD is in a high-energy state compared to the open form (Lahoud et al., 2011), indicating that the trefoil-knot is dynamically constrained. In crystal structures of SPOUT methyl transferases in complex with AdoMet or derivatives, including that of YbeA (Koh et al., 2017), the methyl donor is consistently bound in the high-energy bent form, indicating a paradigm uniquely associated with the trefoil-knot that is required for substrate binding.

By untying the knot, Ko et al. showed that CP74 not only loses the AdoMet binding capacity, but also loses the conformational constraint associated with the knot and becomes more relaxed and more flexible at local dynamics relative to the trefoil-knot-containing native enzyme. Upon thermal denaturation, CP74 unfolds at a lower temperature and proceeds with more intermediates than observed for the native enzyme, indicating a reduced cooperativity. Upon urea denaturation, CP74 requires higher concentrations of the denaturant, consistent with the reduced cooperativity, and it unfolds with much slower kinetics during the phase that needs to unravel the extensive dimer interface as a result of domain-swapping. Once CP74 unfolds from the domain-swapped dimer to a monomeric intermediate, its folding and unfolding into the denatured state is as robust as the knotted native enzyme. The implication from these analyses that CP74 has a reduced cooperativity in folding and unfolding is interesting. The evolution of cooperativity in protein folding transitions is closely linked to the evolution of function as well as the propensity of protein aggregation. A large activation energy barrier in a fully cooperative transition can provide the kinetic control necessary to prevent accumulation of partially unfolded

intermediates, which may promote aggregation. This consideration emphasizes another functional importance of the knot in facilitating the coordinated folding and unfolding of protein.

The successful untying of the knot in YbeA by Ko et al. using circular permutation raises the question of whether the same strategy can be taken to re-create the knot from the unknotted form. This would be a direct test of the previous bioinformatics prediction that the formation of a protein knot arose from an unknotted protein in evolution by circular permutation (Tkaczuk et al., 2007). It would also facilitate protein engineering to create “designer” knotted proteins. Using CP74 as a model, the circular permutation design server CPred has identified potential sites that can be introduced to close off the opening site at position 74 and to re-open the structure in a way different than that of the native enzyme. One of these new sites is at position Gly126 (Figure 1D), which is located in a C-terminal loop of CP74 far away from the knotting loop between β2 and α3, and is at a position not predicted to disrupt the folding of the original knot structure. It will be of great interest to determine whether the opening of position 126, or one of the other predicted sites in the Ile74-sealed CP74, will generate a knot that restores the AdoMet-binding site of YbeA. Possibly, iterative cycles of circular permutation, experimental investigation, and structural analysis and interpretation, are necessary to achieve this goal. Nonetheless, if successfully achieved, the impact is high. It will provide not only a framework to understand how the protein knot is formed, but also an invitation to protein design to integrate the knot structure for novel utility in biotechnology.

Acknowledgments

We thank the support of NIH grants GM114343, GM126210, and AI139202 to YMH for this work.

Author Contributions

All three authors discussed the conceptual framework of the work. SY generated Figure 1, BD used the CPred to predict new sites for re-knotting, and YMH wrote the paper.

Competing Financial Interests

The authors declare no competing financial interests for this work.

References:

Christian, T., Lahoud, G., Liu, C., Hoffmann, K., Perona, J.J., and Hou, Y.M. (2010). Mechanism of N-methylation by the tRNA m1G37 methyltransferase Trm5. RNA *16*, 2484-2492.

Christian, T., Sakaguchi, R., Perlinska, A.P., Lahoud, G., Ito, T., Taylor, E.A., Yokoyama, S., Sulkowska, J.I., and Hou, Y.M. (2016). Methyl transfer by substrate signaling from a knotted protein fold. Nat Struct Mol Biol *23*, 941-948.

Goldenberg, D.P., and Creighton, T.E. (1983). Circular and circularly permuted forms of bovine pancreatic trypsin inhibitor. J Mol Biol *165*, 407-413.

Ko, K.T., Hu, I.C., Huang, K.F., Lyu, P.C., and Hsu, S.D. (2019). Untying a Knotted SPOUT RNA Methyltransferase by Circular Permutation Results in a Domain-Swapped Dimer. Structure.

Koh, C.S., Madireddy, R., Beane, T.J., Zamore, P.D., and Korostelev, A.A. (2017). Small methyltransferase RlmH assembles a composite active site to methylate a ribosomal pseudouridine. Sci Rep *7*, 969.

Lahoud, G., Goto-Ito, S., Yoshida, K., Ito, T., Yokoyama, S., and Hou, Y.M. (2011). Differentiating analogous tRNA methyltransferases by fragments of the methyl donor. RNA *17*, 1236-1246.

Lo, W.C., Wang, L.F., Liu, Y.Y., Dai, T., Hwang, J.K., and Lyu, P.C. (2012). CPred: a web server for predicting viable circular permutations in proteins. Nucleic Acids Res *40*, W232-237.

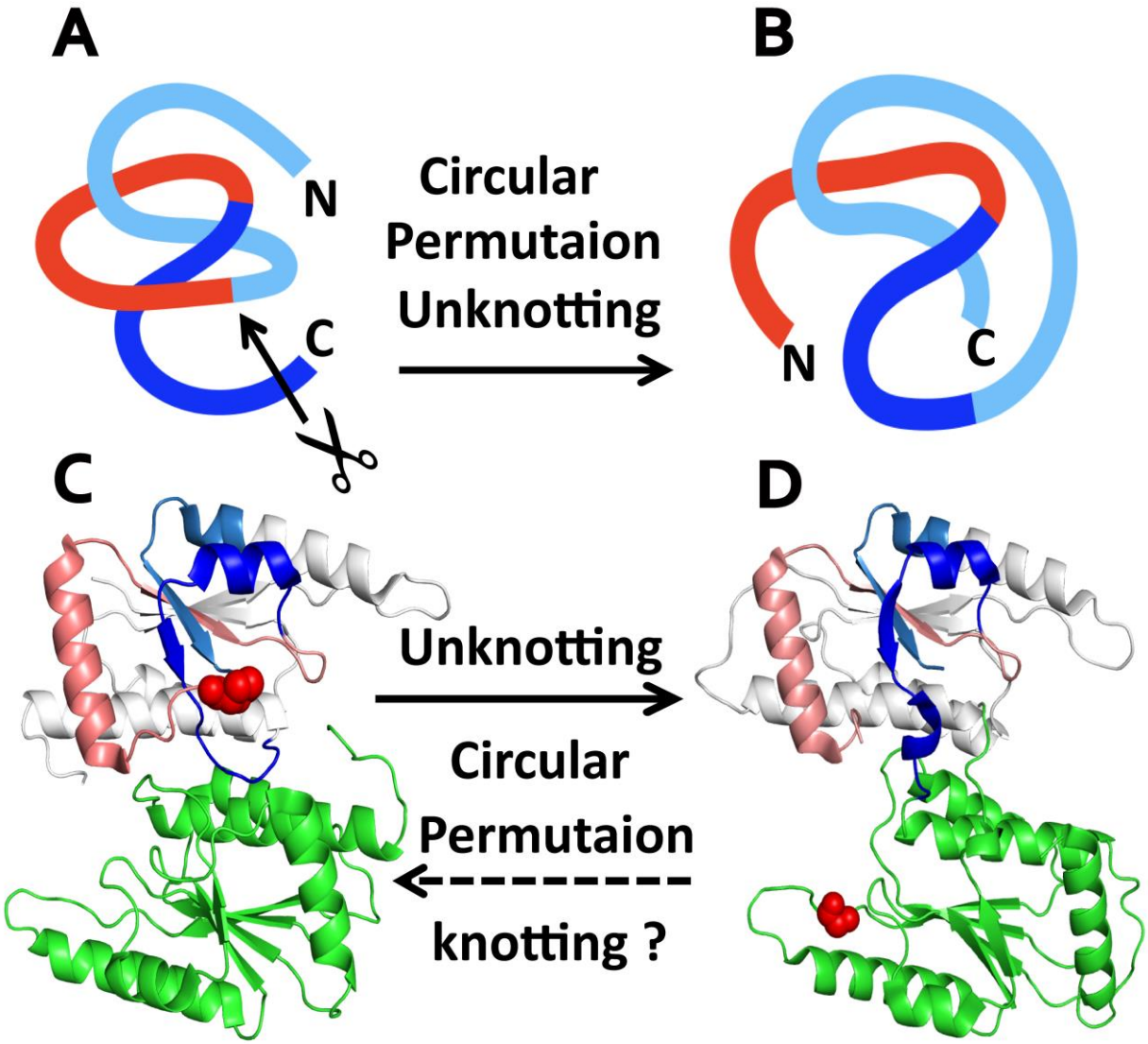
Mallam, A.L., Rogers, J.M., and Jackson, S.E. (2010). Experimental detection of knotted conformations in denatured proteins. Proc Natl Acad Sci U S A *107*, 8189-8194.

Purta, E., Kaminska, K.H., Kasprzak, J.M., Bujnicki, J.M., and Douthwaite, S. (2008). YbeA is the m3Psi methyltransferase RlmH that targets nucleotide 1915 in 23S rRNA. RNA *14*, 2234-2244.

Tkaczuk, K.L., Dunin-Horkawicz, S., Purta, E., and Bujnicki, J.M. (2007). Structural and evolutionary bioinformatics of the SPOUT superfamily of methyltransferases. BMC Bioinformatics *8*, 73.

Figure legend

Figure 1. Knotting and unknotting of a protein by circular permutation. (A and B) Cartoon representation of introducing a circular permutation site to the knotting loop of a trefoil-knot to untie the knot. (C) The native knotted structure of YbeA (PDB:INS5), showing position Ile74 to which a circular permutation opening was created to generate the unknotted CP74. (D) The structure of CP74 (PDB:5ZY0), showing position Gly126 for a new round of circular permutation that may restore the knot. In (C) and (D), the carbon atoms of Ile74 in YbeA and Gly126 in CP74 are highlighted in red.


A
B
N
C
Circular
Permutaion
Unknotting
N
C
C
D
Unknotting
Circular
Permutaion
knotting ?